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TOXIC HAZARD TO THE RABBIT FROM DIRECT AND VAPOR CONTACT WITH HD-CONTAMINATED PLEXIGLAS, CONCRETE, OR XM40 NYLON CARRIER MATERIAL

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The hazard from contact with Plexiglas, concrete, or XM40 nylon carrier material contaminated with mustard (HD) was investigated using the rabbit as the biological model. Contamination levels of 25, 5, and 0.5 mg of neat HD were evaluated both before and after rinsing with the solvent isopropyl alcohol. Both direct contact and vapor contact were evaluated after contact time of 15 or 60 min. Contaminated surfaces were aged for 15 min or 5 hr after solvent rinse-removal. Chemical analyses were done on all test plates. Results indicate that Plexiglas does not retain significant amounts of HD after rinsing with solvent. Concrete retains significant amounts of HD even after rinsing with solvent and aging for 5 hr. The XM40 nylon carrier material retains little HD after solvent rinsing. HD-contaminated concrete is a hazard by direct contact or vapor contact, with little difference in the effect from either contact type. Solvent soaking of HD-contaminated concrete for up to 168 hr does not totally remove the HD. HD-contaminated concrete tests indicate that HD transfer is (continued on reverse)

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19. Abstract (continued)

mainly a vapor transport phenomenon since skin injury was similar from direct contact or vapor contact.

*Formerly assigned to the U.S. Army Chemical Research, Development and Engineering Center.

PREFACE

The work described in this report was authorized under Project 1L162706A553A, Chemistry and Effects of Threat Agents. This project was started in June 1984 and completed in October 1984. Experimental data are contained in laboratory notebook 84-0126.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Chemical Research, Development and Engineering Center (CRDEC), which oversees the use of laboratory animals by reviewing for approval all CRDEC research protocols requiring laboratory animals. This project was assigned LAURC protocol number 21084000A160.

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The authors wish to thank Leonard J. Szafraniec, Gaston E. Dudley, and David I. Rossman, CRDEC, for their assistance as agent custodians and Linda L. Szafraniec for the chemical analysis of the test sample relating to this project. William M. Sayler is also recognized for the many hours he devoted to the preparation of the concrete plates used in this experiment.

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TOXIC HAZARD TO THE RABBIT FROM DIRECT AND VAPOR CONTACT WITH HD-CONTAMINATED PLEXIGLAS, CONCRETE, OR XM40 NYLON CARRIER MATERIAL

1. INTRODUCTION

In recent years, there has been considerable research on chemical agent hazards from contact with surfaces that are supposed to be clean. In 1981, studies[1] assessed the hazard from contact with residual mustard (HD) that could sorb into and subsequently desorb from polyurethane or alkyd-painted steel test plates. The studies indicated that penetration of the painted steel by HD was possible, and that rinsing with a solvent could not completely remove the contamination from the alkyd paint. Subsequent studies[2] with VX and painted steel test showed similar results. Previous investigations indicate[3,4] that surfaces which appear clean can, in fact, contain contamination capable of causing a physiological response in an individual.

Sidman and co-workers at Arthur D. Little proposed an absorption/desorption model predicated on the assumption that agent desorbs from a surface in vapor phase and, in turn, is sorbed by the contacting surface.[5] Another model proposed by Joel Klein (The Models for Sorption and Desorption of Liquid Agents-Their Implications, Unpublished Data, July 1986) assumes that partitioning of the agent between the contaminated surface and the contacting layer may contribute to the transfer process. In this model, it is postulated that the contaminated surface can be compared to a pseudo-liquid. The agent transfers across the interface to the contacting layer, as between two immiscible liquids in contact, at a rate higher than that for vapor transfer.

A toxicological test was designed to evaluate the above models. Animals were exposed to agent vapor and to direct contact with surfaces contaminated with HD. If the toxicological response is the same from direct contact as from vapor, then only vapor transfer is significant. However, if damage is greater from direct contact, then the second model, to include partitioning of the pseudo-liquid, is needed to explain contact hazard.

These tests used distilled HD as the contaminant and rabbits as the test animals. Rabbits were selected since they are the animal-of-choice for skin toxicity studies. The surfaces used in this study were Plexiglas (a low density polyethylene sheeting), concrete, and the XM40 nylon carrier material. Plexiglas was selected because it is a low sorbency material, concrete because it is a high sorbency material, and the XM40 carrier material because it is nylon and represents a cloth substance.

The objective of this study was to identify the exposure hazard.

2. TEST MATERIALS AND ANIMALS

2.1 Chemical Agent.

The distilled HD was obtained from the Chemical Agent Storage Yard. The chemical purity of the distilled HD was 97.9 to 98.7% by nuclear magnetic resonance, and the density was 1.27 gm/ml at 25 °C. All tests were conducted by adjusting dose levels of the agent to its density.

2.2 Test Plates.

2.2.1 Concrete Test Plates.

Concrete test plates were made from a commercial brand of Sakrete-Sand Mix purchased in 80-pound bags, Lot No. 78131-1, code 01-C. To make the test plates, 5 1/2 quarts of water were added to each bag of mix, the mixture poured into molds, and the cured concrete cut into plates 1 inch x 2 inches x 0.75 inch thick.

2.2.2 Plexiglas Test Plates.

Plexiglas plates (1 inch x 2 inches) were cut from commercially purchased 1/8-inch thick sheets of polymethyl methacrylate.

2.2.3 XM40 Nylon Carrier Material.

Nylon material was obtained from the Physical Protection Directorate, Chemical Research, Development and Engineering Center (CRDEC). The material was cut into pieces 1 inch x 2 inches.

2.3 Stainless Steel Template.

A stainless steel template was used to suspend the test plates 1 cm above the test area on the rabbit's back for vapor contact as previously described.[1]

2.4 Rabbits.

A total of 360 adult male and/or female New Zealand White rabbits (1.8 to 3.6 Kg), commercially raised and procured by the U.S. Army Medical Research Institute of Chemical Defense (USAMRICD), were used in these tests. The animals were quarantined and certified healthy prior to issue. Six rabbits were used in each test group.

2.5 Solvents.

Solvents used in this experiment were obtained from the following sources:

- Isopropyl Alcohol, Reagent A.C.S., Lot OHNC, Mallinckrodt Inc., Science Products Division, St. Louis, MO.

- Diethyl phthalate (DEP), 98% minimum purity. MCB Manufacturing Chemist, Inc., Associate of G. Merek, Darmstadt, Germany, 2909 Highland Avenue, Cincinnati, OH.

2.6 Syringes.

Syringes used to deliver accurate doses of HD were as follows:

- Syringes, numbers D6181 and C3960, 0.5 ml, calibrated to deliver HD at a rate of 5.43 micrometer divisions/milligram.

- Syringe, number X8884, 0.25 ml, calibrated to deliver HD at a rate of 9.448 micrometer divisions/milligram.

- A mechanical Agla-micrometer-driven syringe holder designed and used for delivering precise amounts of a liquid in either microgram or milligram quantities was manufactured by Burroughs Welcome Co., England. This syringe holder was used throughout the course of these tests to ensure accurate HD delivery.

3. TEST METHODS AND RESULTS

3.1 Test Animal Preparation and Handling.

Rabbits were prepared by clipping their dorsal area free of hair 18 hr before testing. Clipping was done with standard small animal clippers equipped with a number 2 blade, followed by a second clipping with a number 40 blade. All rabbits were indentified by ear tag, weighed, sexed, and numbered sequentially from 1 to 360.

On test day, each rabbit was placed in a metal stanchion which restrained the animal by a collar around the neck. Rabbits, in groups of six, were placed in laboratory-filtered fume hoods with a face velocity of 150 + 30 linear feet per minute. They were returned to their home cages after the 24-hr test procedure. Prior to their return to the home cage, the clipped areas of all animals were observed and graded for skin irritation. Following the initial 24-hr irritation reading, all rabbit skin test sites were wiped with isopropyl alcohol to remove residual HD surface contamination.

3.2 Skin Irritation Evaluations.

Irritation to rabbit skin was evaluated and scored according to procedures outlined in Code of Federal Regulations, 16 CFR 1500:41[6] and according to the Draize technique.[7] Evaluations were done at the time of test plate removal and at 24, 48, and 72 hr. Irritation was scored according to procedures indicated in Table 1. The final irritation score represented an average of the six rabbit values for intact skin at both 24 and 72 hr after compound application. A final primary irritation score of 5 is indicative of primary skin irritant; 2 to 4.99, a moderate skin irritant; 0.01 to 1.99, a

mild skin irritant; and 0.00 indicates substance is nonirritating to rabbit skin.

Table 1. Procedures for Scoring Skin Irritation

Skin Reaction	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formations (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

At the 72-hr evaluation, the size of the area of irritated skin was measured to the nearest 0.125 inch; and the area of damage for eschar formation, erythema, and edema were recorded for each rabbit. The average of the damaged area for each group of six test animals was recorded for comparative purposes.

3.3 Test Plate Contamination Procedure.

Test plates of Plexiglas, concrete, and XM40 nylon cloth, 1 inch x 2 inches, were contaminated with single droplets of HD that were dispensed with a calibrated syringe and an Agla-micrometer syringe holder. Drop weights of agent used were 25 mg, 5 mg, or 0.5 mg.

3.4 Test Plate Aging Procedure.

All HD-contaminated test plates were aged for 30 min in a fume hood before use in contact procedures.

Absorbent towels were placed under the plates to prevent contamination of the hood surface. Plates were tested on rabbits' backs by either direct or vapor contact. Additional plates used as controls did not contact rabbit skin.

3.5 Test Plate Contamination Removal Procedure.

HD was removed from certain test plates by isopropyl alcohol dispersed from large syringes through 16-gauge, stainless steel needles. Slight downward hand pressure was applied to the syringe plunger, and solvent was dispersed in a sweeping motion from top to bottom and back and forth over the entire plate. The solvent and the HD removed were washed into a decontamination container within a filtered fume hood. Agent rinse-removal volumes are shown in Table 2.

Table 2. HD - Isopropyl Alcohol Rinse-Removal Volumes*

HD Droplet Size (mg)	Isopropyl Alcohol Volume (ml)	Approximate Dispersal Time (sec)
25.0	35	30
5.0	20	15
0.5	15	10

*These agent removal procedures and solvent volumes were prescribed by the Physical Protection Directorate, CRDEC.

3.6 Test Plate Aging Time Following Agent Rinse-Removal.

The worst-case test plates were rinsed to remove HD, wafted until the solvent dried (about 30 sec), and immediately placed on test animals. Other test plates were rinsed to remove HD and placed, contaminated side up, in the fume hood. These plates aged for an additional 15 min or 5 hr, depending upon test design, prior to contact with rabbit skin.

3.7 Test Plate Application Procedure and Contact Time with Animals.

Test plates were applied to rabbits' skin for either 15 or 60 min by the following two procedures.

3.7.1 Direct Contact.

By this procedure, the contaminated test plate was attached, contaminated side down, to the clipped area of the rabbit's skin. A piece of refrigeration tape was placed over the plate to attach the plate to the rabbit. An additional seal of polyethylene film was tape-sealed over the taped plate to form an additional seal to prevent loss of agent to the atmosphere.

3.7.2 Vapor Contact.

In this procedure, the stainless steel template, described in a previous report,[1] was tape-attached to the clipped skin of the rabbit. The contaminated test plate was then affixed to the template (contaminated side down and facing the skin) by a refrigeration tape seal, which suspended the plate 1 cm above the skin surface. This entire apparatus was then sealed with polyethylene film and tape-sealed to the rabbit to prevent loss of agent vapor.

3.8 Test Plate Removal.

All test plates were analyzed chemically for residual agent contamination. The test plates were removed from the rabbits, following the required contact time, and placed immediately into DEP. The plates were allowed to soak for up to 192 hr prior to analysis for residual agent content.

3.9 Chemical Analysis Procedure.

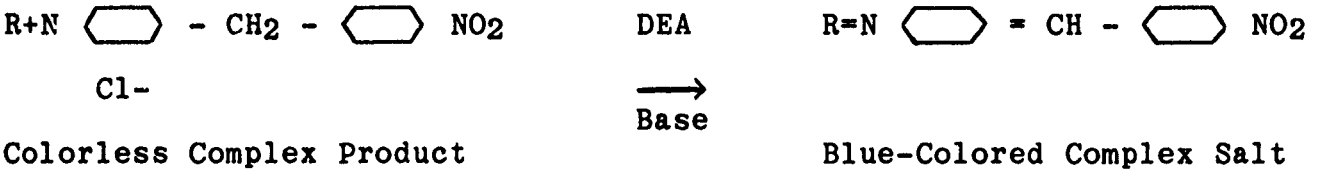
Chemical analyses of materials tested were done by personnel of the Research Directorate, CRDEC.

HD is easily detected and identified using a colorimetric method.[8] The main reagent used in this test is 4-(p-nitrobenzyl)pyridine (DB_3). DB_3 reacts slowly with HD at ordinary temperatures, but at elevated temperatures (70 °C or higher) DB_3 reacts rapidly to form an additional product. This product reacts with diethylamine to form an intense blue color which constitutes the test for HD. Test analyses were run using a Technicon AutoAnalyzer II Colorimeter with the results recorded on a strip chart recorder.

DEP was selected as the solvent because of the high degree of stability of HD in DEP. The absorption of HD in DEP is so complete that concentrations as low as 0.004 µg/ml can be detected with the AutoAnalyzer II. During this test, the range of detection was set for 0.1 to 6 µg/ml.

The following schematic describes the reactions involved:

$$(Cl\ CH_2\ CH_2)\ S + Ni\ (CH)_2 : C\ (CH_2\ C_6H_4NO_2)\ CH : CH \xrightarrow[\text{Heat}]{93\ ^\circ C} (DB_3)$$



Colorless Complex Product — Blue-Colored Complex Salt

The analysis system was calibrated with a series of HD standards ranging from 0.38 to 5.75 µg/ml. To check if the Plexiglas or concrete interfered with the HD analysis system, samples of Plexiglas were soaked in a series of HD standards for 48 hr; concrete samples were soaked for 24 hr. The test indicated that Plexiglas did not effect the concentration of HD. The results of the concrete-HD test indicated a 2% decrease in the amplitude of the chart peaks. This decrease was considered to be negligible in regard to the final test results.

A preliminary analysis of contaminated concrete plates showed a very low and nonuniform HD recovery rate of 0.6 to 35%. To correct this low yield, the plates were soaked in solvent for 168 hr prior to analysis. Some of the concrete plates were removed and placed, one at a time, in a stainless steel beaker. Using a heavy steel rod, the concrete was struck repeatedly until crushed. The crushed material was placed in a jar containing 50 ml of DEP for 24 hr. This sample was then filtered and analyzed for HD content. Analysis of the filtered solution indicated that about 50% of the HD was still trapped in the concrete after 168 hr of soaking.

From the above preliminary tests, the following schedule of soak times in the DEP solvent were used:

- Plexiglas - soaked for 24 hr prior to analysis.
- XM40 nylon - soaked for 24 hr prior to analysis.
- Concrete - soaked for 168 hr; some of the samples were crushed and soaked an additional 24 hr prior to analysis.

3.10 Test Schedule and Results.

A summary of the test phases and conditions are given in Table 3.

Table 3. Test Phases in HD Contact Hazard Study

Test Phase	Aging Time (min)	Solvent Rinse	Post-Rinse Aging (min)	Rabbit Contact Time (min)
I	30	no	no	60
II	30	yes	no	60
III	30	yes	15	60
IV	30	yes	15	15
V	30	yes	300	60
VI	30	yes	300	15

NOTE: Plexiglas was run in Test Phase I and II only. XM40 nylon was run in Test Phase II and III only. Concrete was run in all the Test Phases.

3.10.1 Test Plate Control Irritation Test.

To confirm that the Plexiglas and concrete test plates did not cause skin irritation in rabbits, a total of six rabbits were prepared and tested according to procedures described earlier. A volume of 0.025 ml of distilled water was applied to the skin of rabbits, and one test plate of each type (Plexiglas or concrete) was taped to the clipped area of the rabbit's skin for 60 min. Following the 60-min contact time, the plates were removed and skin surfaces were evaluated for both erythema and edema at 60 min, 24, 48, and 72 hr.

The results of this test showed no skin irritation developing from either test plate type following 60 min of direct contact time. A contact time of 60 min was chosen, rather than 24 hr as described in 16 CFR 1500.41, because 60 min would be the longest contact time for test plates during the actual agent tests. Based on these results, investigators determined that any irritation observed in later tests would be directly related to the HD contamination.

3.10.2 Control Test, HD on Plexiglas Test Plates, 30-Min Age, No Rinse-Removal, and 60-Min Contact.

A worst-case control test was done to assess the maximum damage that could be expected to occur to rabbit skin from HD applied to Plexiglas test plates in doses of 25, 5, or 0.5 mg. Fifty-four contaminated test plates were aged for 30 min in fume hoods. Following the 30 min age, 36 plates (6 per dose and contact type) were

placed in contact with rabbit skin for 60 min by the direct contact or vapor contact procedure. Following contact, test and control plates (6 control plates per dose level) were placed into DEP solvent for chemical analysis.

The results of these tests, shown in Tables 4 and 5, indicate that all three dose levels of HD, by both exposure methods, are severely irritating to rabbit skin, and all are primary skin irritants (score greater than 5). Most rabbits developed eschar formation and severe edema from all three doses.

Table 4. Contact Hazard - Plexiglas Plates

	Amount of HD Applied to Plates - (mg) - 24 Hour Solvent Soak								
	25.0			5.0			0.5		
Test Conditions	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.*	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.
Direct Contact									
HD-30 min age no rinse 60 min contact	15.3 (61.3%)	12.96	7.75	3.4 (68.3%)	4.13	7.92	0.136 (27.2%)	2.38	7.75
Vapor Contact									
HD-30 min age no rinse 60 min contact	12.2 (48.9%)	10.54	7.33	4.0 (79.7%)	8.67	6.92	0.096 (19.1%)	3.69	6.54
Direct Contact									
HD-30 min age rinsed 60 min contact	Below** Detection Limits	0	0	Below Detection Limits	0	0	Below Detection Limits	0	0
Vapor Contact									
HD-30 min age rinsed 60 min contact	Below Detection Limits	0	0	Below Detection Limits	0	0	Below Detection Limits	0	0

* P.I.I. = Primary Irritation Index
** Detection Limit Less Than 0.0016 mg/sample

Table 5. Contact Hazard - Control Plexiglas Plates

	Amount of HD Recovered from Plates After 24 Hour Soak (mg)		
	Amount of HD Applied to Plates (mg)		
Test Conditions	25.0	5.0	0.5
HD - 30 min age, no rinse	25.6 (102.5%)	4.6 (92.7%)	0.304 (60.7%)
HD - 30 min age, rinsed-no age	Below Detection Limits*		

*Detection limits less than 0.0016 mg/sample.

Table 4 shows the average area (square inches) of skin damage, in the form of edema, resulting from these tests. The area of skin damage was larger for the 5 and 0.5 mg dose levels from vapor contact than by direct contact at the same dose levels due to the spreading of the vapors. The irritation scores for skin damage were greater by the direct contact method due to the smaller, more concentrated area of agent contact.

After removal from rabbits, all test plates and the control plates were analyzed for residual HD contamination. The results are shown in Tables 4 and 5. Table 5 shows that the 25 and 5 mg control plates, without rinsing, retained the most (93 to 100%) HD, while the 0.5 mg control plate retained about 61% of the original HD. The missing HD is assumed lost by vaporization during the 30-min age period in the hood. Table 4 shows that, after subtracting HD lost from control plates, 25 to 39% of the HD was transferred to the rabbit skin by direct contact and 13 to 51% by vapor contact. HD transfer is postulated since the degree of skin irritation observed was considerable.

3.10.3 HD on Plexiglas Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, 60-Min Contact.

Plexiglas was chosen as a test surface because the sorption of HD into the surface would occur very slowly. Thus, the HD would remain on or near the surface and most of the contamination should be removed with the isopropyl alcohol rinse. Transfer of the agent by direct contact should produce skin irritation on the rabbits, while vapor contact should produce much less irritation. HD was applied to Plexiglas plates as 25, 5, or 0.5 mg droplets (18 plates per dose) and allowed to age and spread on the plates for 30 min. Following the aging period, the HD contamination was rinsed off the plates. The plates were wafted dry, and six plates of each dose were placed in direct or vapor contact with rabbit skin for 60 min. Following contact, these plates, along with the control plates (6 per dose), were placed in DEP solvent for analysis.

The results of this test show no skin irritation observed on any of the 36 rabbits tested during the 72-hr observation. Table 4 shows that no skin irritation resulted from any of the three doses by either direct contact or vapor contact procedures.

Results of the chemical analyses of the test plates are given in Tables 4 and 5. Table 5 indicates that more than 99.7% of the HD on the control plates was removed by the rinse. This nearly complete rinse-removal of HD was confirmed when plates tested on rabbit skin produced no observable skin irritation (Table 4). This is also confirmed by the chemical analyses of the rabbit test plates which indicated residual HD contamination below the detection limits (0.0016 mg/sample) of the chemical analysis test (Table 4).

Prior to rinsing with isopropanol, HD had spread very little over the Plexiglas test plates, and the plates remained wet. A best visual estimate of the HD spread (area percent of plate) on the plates was made and is recorded in Table 6.

Table 6. HD Spread on Plexiglas Test Plates Following 30-Min Age in Filtered Fume Hood

Dose HD (mg)	Mean Area Plate Covered* (%)	Range (%)
25.0	11.1	10-12.5
5.0	3.2	3-4
0.5	1.0	None

*Mean spread for 36 plates

3.10.4 HD on Concrete Test Plates, Aged 30 Min, No Rinse-Removal, and 60-Min Contact.

This worst-case control test involved applying 25, 5, or 0.5 mg of HD to 12 concrete plates each, aging the plates for 30 min, and placing them in direct or vapor contact with rabbit skin for 60 min. Six control plates of each dose were tested concurrently. It was noted that the HD did not spread out over the surface but soaked into the concrete very rapidly. All plates were chemically analyzed for residual contamination following completion of the test.

The results of this test (Tables 7, 8, and 9) indicate that all three dose levels of HD, by both contact methods, are severely irritating to rabbit skin, and all are primary skin irritants (scores greater than 5). Most rabbits developed eschar formations, necrotic skin, and severe edema from all doses. The area of skin damage was generally larger from vapor contact than from direct contact due to the spread of the HD vapors within the confines of the template.

The results of the chemical analyses varied with the length of time the plates were soaked in DEP (72 to 168 hr). After 168 hr of soaking in solvent, selected plates were crushed to liberate trapped HD and reanalyzed after soaking in additional DEP. The longer soak time, 168 hr compared to 72 hr, increased the amount of HD recovered about four times. The crushing of the concrete increased the recovered HD two times in comparison to that recovered

Table 7. Contact Hazard - Control Concrete Plates

Test Conditions	Percent of HD Recovered from Plates Amount of HD Applied to Plates (mg) 25.0		5.0		0.5	
HD-30 min age, no rinse (60 min contact)	(168 hr)* 32.5 (soaked)	62.9 (crushed)	11.7 80.2 (crushed)	(72 hrs) (168 hrs)	9.7 54.0 (crushed)	(72 hrs) (168 hrs)
HD-30 min age, rinsed (60 min contact)	51.3	(192 hrs)	52.2	(192 hrs)	32.1	(192 hrs)
HD-30 min age, rinsed 15 min age (60 min contact)	31.0	(192 hrs)	21.3	(192 hrs)	13.4	(192 hrs)
HD-30 min age, rinsed 15 min age (15 min contact)	29.6	(168 hrs)	33.4	(168 hrs)	22.1	(168 hrs)
HD-30 min age, rinsed 5 hr age (60 min contact)	14.05	(168 hrs)	9.97	(168 hrs)	6.37	(168 hrs)
HD-30 min age, rinsed 5 hr age (15 min contact)	13.84	(168 hrs)	11.48	(168 hrs)	12.80	(168 hrs)

*Hours of solvent soak

Table 8. Contact Hazard - Concrete Plates - Direct Contact

Test Conditions	Amount of HD Applied to Plates (mg) 25.0 % HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.*	5.0 % HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.	0.5 % HD Recovered	Edema Area Mean (fq. In.)	P.I.I.
HD-30 min age no rinse 60 min contact	15.2 (72 Hr.)**	3.13	7.59	(72 Hr.) 11.4 (168 Hr.) 59.0	3.17	7.91	(72 Hr.) 7.7 (168 Hr.) 32.0	0.54	7.75
HD-30 min age rinsed 60 min contact	46.7 (192 Hr.)	3.64	7.83	31.1 (192 Hr.)	1.23	7.92	26.3 (192 Hr.)	0.42	7.09
HD-30 min age rinsed-15 min age 60 min contact	24.6 (192 Hr.)	2.28	7.84	20.0 (192 Hr.)	1.00	7.92	18.5 (192 Hr.)	0.35	7.92
HD-30 min age rinsed-15 min age 15 min contact	28.8 (168 Hr.)	2.09	7.42	22.7 (168 Hr.)	1.10	8.00	16.0 (168 Hr.)	0.18	6.58
HD-30 min age rinsed-5 hr age 60 min contact	11.5 (168 Hr.)	2.62	7.75	8.8 (168 Hr.)	1.14	7.75	4.3 (168 Hr.)	0.33	5.84
HD-30 min age rinsed-5 hr age 15 min contact	11.8 (168 Hr.)	1.09	7.55	8.3 (168 Hr.)	0.17	7.25	11.9 (168 Hr.)	0.00	0.00

* P.I.I. = Primary Irriation Index
**Hours of solvent soak.

Table 9. Contact Hazard - Concrete Plates - Vapor Contact

Test Condition	Amount of HD Applied to Plates (mg)								
	25.0			5.0			0.5		
	% HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.*	% HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.	% HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.
HD-30 min age no rinse 60 min contact	15.1 (72 Hr.)**	7.38	7.79	13.0 (72 Hr.) 55.5 (168 Hr.)	5.14	7.71	6.7 (72 Hr.)	0.41	6.25
HD-30 min age rinsed 60 min contact	51.8 (192 Hr.)	5.76	7.79	58.7 (192 Hr.)	3.64	7.67	28.8 (192 Hr.)	0.20	4.58
HD-30 min age rinsed-15 min age 60 min contact	29.4 (192 Hr.)	4.63	7.67	24.5 (192 Hr.)	5.19	7.71	18.7 (192 Hr.)	0.57	5.71
HD-30 min age rinsed-15 min age 15 min contact	35.6 (168 Hr.)	6.54	7.84	24.1 (168 Hr.)	3.87	7.42	21.0 (168 Hr.)	0.10	2.00
HD-30 min age rinsed-5 hr age 60 min contact	14.9 (168 Hr.)	5.76	6.79	10.9 (168 Hr.)	1.28	6.12	4.5 (168 Hr.)	0.00	0.00
HD-30 min age rinsed-5 hr age 15 min contact	10.4 (168 Hr.)	0.74	7.42	8.5 (168 Hr.)	0.00	0.00	13.0 (168 Hr.)	0.00	0.00

* P.I.I. = Primary Irritation Index
** Hours of solvent soak.

by simply soaking the concrete in DEP. The total amount of HD recovered after contact ranged from 32% in the 0.5 mg plates to 59% in the 5 mg plates. There was possibly more HD trapped in the concrete, but the extra effort required to recover it was not done.

3.10.5 HD on Concrete Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, and 60-Min Contact.

The second concrete test involved similar dosing of 54 plates with HD as before, aging for 30 min, rinsing with isopropyl alcohol, and immediate contact with rabbit skin for 60 min by both direct contact and vapor contact. All 36 test plates, along with 18 control plates, were analyzed for residual HD contamination after soaking in DEP for 192 hr.

The resulting skin irritations are shown in Tables 8 and 9. Rinsing showed only minimal lessening of skin edema or skin irritation except at the 0.5 mg dose of vapor contact. The irritation scores, except as noted, still indicate that enough HD remained in the concrete to cause primary skin irritation.

Chemical analyses were performed after soaking the plates in solvent for 192 hr. The results are shown in Tables 7, 8, and 9. This additional soaking time produced results similar to those produced previously by the shorter soaking time combined with crushing the concrete plates. The results indicate that rinsing removed only a minimal amount of HD since 26 to 59% of the original HD was still in the plates after rabbit contact. This is about the same, 32 to 52%, as remained on the control plates (Table 7).

3.10.6 HD on Concrete Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, Aged 15 Min, and 60-Min Contact.

This test was designed to determine if additional aging after rinse-removal of HD would remove additional residual HD contamination from the concrete test plates. A total of 54 concrete plates were contaminated with the three doses of HD (18 plates each dose) and aged for 30 min in the fume hood. After aging, the plates were rinsed with isopropyl alcohol, allowed to age an additional 15 min, and six plates of each dose were placed in either direct contact or vapor contact with rabbit skin for 60 min. Following contact, these plates, along with 18 control plates, were placed in solvent for 168 hr and chemically analyzed for residual HD.

The results of this test are shown in Tables 7, 8, and 9. The extra 15 min aging after rinsing reduced the amount of HD remaining in the plates from 8 to 35% (Tables 7-9). However, enough HD remained in the plates to cause severe skin irritation in all rabbits. This irritation was similar in nature to that caused by a primary skin irritant. The edema area for direct contact was slightly smaller than in the previous test but about the same for vapor contact when compared to the previous test (Tables 8 and 9).

The results of the chemical analyses are shown in Tables 7, 8, and 9. All three tables show a decrease in the amount of HD found in the plates as compared to the previous test. The control plates contained only 15 to 31% residual HD as compared to 32 to 52% HD in the previous test. The contact plates showed similar results, 18 to 29% recovered HD as compared to 26 to 59% in the previous test. This indicates that some additional HD was lost by evaporation during the extra 15 min of aging following rinsing but not enough to eliminate the contact hazard.

3.10.7 HD on Concrete Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, Aged 15 Min, and 15-Min Contact.

This test was designed to determine if a shorter contact time would lessen the skin irritation from HD. A total of 54 concrete plates were contaminated with the three doses of HD (18 plates per dose). The plates were aged for 30 min, rinsed with isopropyl alcohol, aged an additional 15 min in a fume hood before six plates of each dose were placed in either direct contact or vapor contact with rabbit skin for 15 min. Following contact, these

plates, along with 18 control plates, were placed in the solvent for 168 hr and then analyzed chemically for residual HD content.

The results of this test are shown in Tables 7, 8, and 9. Even with the reduced contact time, it was still long enough to cause primary skin irritation with both contact types except at the lowest dose (0.5 mg) of the vapor contact. These similar results were seen in the damaged area in the form of edema. The size of the edema area was in relation to the amount of the original doses for both the direct and vapor contact.

The results of the chemical analysis showed that the contact test plates contained about the same amount of residual HD as the 60 min contact test (Tables 8 and 9). The control plates showed a slight increase in the amount of HD recovered from the lower two doses as shown in Table 7. This difference may be due to problems in removing all the HD from the concrete plates or with differences in concrete composition.

3.10.8 HD on Concrete Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, Aged 5 Hr, and 60-Min Contact.

This test studied the effects of longer aging (5 hr) of the concrete plates after rinse-removal with isopropanol. A total of 54 concrete plates were contaminated with the three doses of HD (18 plates per dose). These plates were aged for 30 min, rinsed with isopropyl alcohol, aged an additional 5 hr in a fume hood, and then six plates of each dose were placed in either direct or vapor contact with rabbit skin for 60 min. These plates, along with 18 control plates, were placed in solvent for 168 hr prior to chemical analysis for residual HD.

The results shown in Tables 8 and 9 indicate that there is enough residual HD remaining in the concrete test plates after rinsing and 5 hr of aging to cause primary skin irritation from all doses with direct contact and with the two higher doses after vapor contact. None of the six vapor contacts at the lowest dose (0.5 mg) caused any skin irritation.

The results of the chemical analysis are shown in Tables 7, 8, and 9. These results show that the additional 5-hr aging reduced the residual HD contamination in the concrete plates by one-fourth to one-half of the content in the previous test with only 15 min aging after rinsing. This reduction is seen in the control plates and in both types of contact plates. The additional 5-hr aging allowed more HD to be removed by evaporation, but enough HD still remained to cause severe skin irritation.

3.10.9 HD on Concrete Test Plates, Aged 30 Min, Isopropyl Alcohol Rinse, Aged 5 Hr, and 15-Min Contact.

In this test, 54 concrete plates were contaminated with the three doses of HD and aged for 30 min in a fume hood. After

aging, they were rinsed with isopropyl alcohol, aged an additional 5 hr, and six plates from each dose were placed in either direct or vapor contact with rabbit skin for 15 min. Eighteen control plates were run concurrently. All plates were placed in solvent for 168 hr prior to chemical analysis for residual HD.

The results of this 15-min contact time (Tables 8 and 9) indicate that enough HD was transferred to the skin from the 25- and 5-mg direct contact plates and from the 25-mg vapor contact plates to cause primary skin irritation with a score of more than 5. However, the area of edema was reduced by one-sixth to one-half in size by this shorter contact time. There was no skin irritation from the 0.5-mg direct contact plates nor any from the 5- or 0.5-mg vapor contact plates. These results indicate that the shorter contact time, along with the lower HD content, reduced the amount of HD transferred at the lower doses by the vapor procedure and at the lowest dose by direct contact.

The results of the chemical analyses (Tables 7, 8, and 9) indicate that from 8 to 14% of the residual HD remained trapped in the concrete plates after rinsing and aging for 5 hr. This was enough HD to cause serious skin irritation at the higher doses but not enough at the lower doses with this shorter contact time.

3.10.10 HD Spread on Concrete Plates.

Prior to rinsing with isopropyl alcohol, the HD had spread very little over the concrete plates. Instead, HD had soaked deep into the concrete, and the surface was either damp-dry or dry. A best visual estimate of the HD spread and surface condition of the plates is given in Table 10.

Table 10. HD Spread on Concrete Plates Following 30-Min Aging in a Filtered Fume Hood

Dose HD (mg)	Plate Condition (surface)	Mean Area Plate Covered* (%)	Range (%)
25.0	Damp-Dry	14	10-18
5.0	Dry	5	3-8
0.5	Dry	1	None

*Mean spread for 108 plates at each dose.

The studies of HD on concrete plates indicated that most of the HD soaked into the concrete and neither rinsing nor aging for 5 hr removed enough HD to eliminate the contact hazard. This concluded the tests with concrete plates.

3.10.11 XM40 Nylon Carrier Material.

The next test used XM40 nylon carrier cloth which is an experimental material used to make carrier containers for protective masks.

3.10.12 HD on XM40 Test Cloth, Aged 30 Min, Isopropyl Alcohol Rinse, No Age, and 60-Min Contact.

The previous tests with Plexiglas plates show that without rinse-removal the residual HD contamination is a serious contact hazard. Testing with XM40 material started with the rinse-removal step. In this test, 54 XM40 nylon patches were contaminated with the three doses of HD and aged for 30 min in a fume hood. The patches were rinsed with isopropyl alcohol, and six patches from each dose were placed in solvent for 24 hr prior to chemical analysis for residual HD.

The results of the skin irritation test are shown in Table 11. The direct contact patches dosed with 25 mg contained enough HD after rinsing to cause a primary skin irritation with a score of 6.25. The 5-mg dose caused a moderate skin irritation while the 0.5-mg dose caused only a mild irritation. The two higher, direct-contact doses showed a slight amount of edema, while the 0.5-mg dose showed none.

None of the vapor contacts showed any edema formation. The 25-mg dose showed a mild skin irritation, while the two lower doses (5 and 0.5 mg) showed no skin irritation.

The results of the chemical analyses are shown in Tables 11 and 12. The control patches contained from 0.01 to 0.05 mg HD following the rinsing procedure. After rabbit contact, the analysis of the patches showed HD content below the detection limits of 0.0016 mg/ml. This analysis indicates that as much as 0.0077 to 0.0474 mg of HD transferred to the rabbit skin and caused the irritation as seen in Table 11. The analysis of patches from vapor contact with the rabbits also showed HD content below the detection limits of the test, but only the 25-mg patches showed any skin irritation. The rest of the HD was apparently lost by evaporation.

3.10.13 HD on XM40 Test Cloth, Aged 30 Min, Isopropyl Alcohol Rinse, Aged 15 Min, and 60-Min Contact.

In this test, 54 XM40 nylon patches were contaminated with the three doses of HD and aged for 30 min in a fume hood. The patches were rinsed with isopropyl alcohol, aged an additional 15 min, and six patches of each dose were placed in either direct

or vapor contact with rabbit skin for 60 min. Following rabbit contact, these patches and the 18 control patches were placed in solvent for 24 hr prior to chemical analysis for residual HD.

Table 11. Contact Hazard - XM40 Nylon Cloth - Direct and Vapor Contact

	Amount of HD Applied to Plates (mg) - 24 Hour Solvent Soak								
	25.0			5.0			0.5		
Test Condition	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.*	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.	Mg HD Recovered	Edema Area Mean (Sq. In.)	P.I.I.
HD-30 min age, rinsed 60 min direct contact	BDL**	0.024	6.25	BDL**	0.093	2.25	BDL**	none	0.42
HD-30 min age rinsed 60 min vapor contact	BDL	none	0.50	BDL**	none	0.00	BDL**	none	0.00
HD-30 min age, rinsed-15 min age, 60 min direct contact	0.0029 (0.015%)	0.01	0.76	0.0029 (0.062%)	none	0.17	0.0029 (0.583%)	none	0.00
HD-30 min age, rinsed, 15 min age, 60 min vapor contact	0.0048 (0.02[illegible]%)	none	0.00	0.0024 (0.050%)	none	0.00	0.0029 (0.583%)	none	0.00

* P.I.I. - Primary Irritation Index
** B.D.L. - Below detection limit of 0.0016 mg/sample (0.15 µg/ml)

Table 12. Contact Hazard - Control XM40 Nylon Cloth

	Amount of HD Recovered From Cloth After 24 Hour Soak (mg). Amount of HD Applied to Plates (mg)		
Test Conditions	25.0	5.0	0.5
HD-30 min age, rinsed, no age	0.049 (0.20%)	0.014 (0.28%)	0.0093 (1.87%)
HD-30 min age, rinsed, 15 min age	0.0125 (0.050%)	0.0082 (0.167%)	0.0030 (0.607%)

The skin irritation results are shown in Table 11. None of the vapor contact patches produced any skin irritation at any of the three doses. The two higher doses, direct contact patches, produced very mild skin irritation and slight edema at the higher

dose only. The lowest dose (0.5 mg) direct patch produced no skin irritation.

The results of the chemical analyses are shown in Tables 11 and 12. The control patches contained from 0.003 to 0.01 mg of HD following rinsing and 15 min of aging. The direct contact patches contained 0.0029 mg of HD at each dose. The vapor contact patches showed similar HD content at the lower doses and 0.0048 mg in the patches at the 25-mg dose. These contamination densities are near the lower detection limits of the analyzer. This is also shown by the good correlation of skin irritation to the HD content in the patches. This test shows very little HD remaining in the cloth after rinsing and aging. The test indicates some additional HD was lost by evaporation during the 15 min of aging following rinsing.

3.10.14 Status of Rabbits Used in These Tests.

Of the 360 rabbits used in these experiments, all rabbits survived the total test time and were humanely euthanatized with intravenous T-61 following test completion.

4. DISCUSSION

The objectives of this test program and the previous programs[1,2] were to quantitate the contact hazard of contaminated surfaces (by direct contact or vapor contact) and to select a model to describe these processes. In the first study, HD and thickened HD (THD) were used to contaminate alkyd and polyurethane painted steel plates, and it was demonstrated that the transfer of agent by direct contact produced skin injury in the rabbits that was more severe than would have been predicted by extending the vapor contact results.[1] These results were consistent with the partition model. In this study, the model predicts the transfer of agent by both a vapor transfer route and by a partitioning of agent found near the contaminated surface to the contacting skin. Similar results were obtained in the VX study employing painted steel as the test surface.[2]

The surfaces used for this study were chosen to determine whether partitioning is applicable to nonpermeable materials such as the polymethyl methacrylate (PMMA) sheeting or to a highly permeable surface such as concrete. Using the plastic sheet, diffusion of the agent into the surface is very slow, and the agent will be at or near the surface. Concrete, on the other hand, sorbs the agent into the material and the amount of agent near the surface will be low. It would appear that partitioning occurs when using the plastic but does not occur with concrete.

The results for the concrete surfaces, reported in Tables 8 and 9, suggest that the predictions are borne out in practice. The data are consistent with a vapor transport mechanism in both the direct contact and vapor contact cases. The severity of the skin damage observed in the vapor contact experiments had an irritation

index of greater than 6.79 for the 25-mg contamination level which compared to an index of greater than 7.42 for the direct contact experiments. An index of greater than 5 is considered a primary irritation, and the differences observed between the comparable tests are of minor significance. As anticipated, the area of skin injury is greater for the vapor contact. These results are consistent with the assumption that the quantity of agent transferred to the skin was approximately the same in the direct contact and vapor contact experiments. This result is predicted by the vapor transport model. The results obtained at the 5-mg contamination level are also consistent with the vapor transport mechanism. At the 0.5-mg contamination level, the alcohol rinse process removes sufficient agent so that the residual, 50-100 µg, is at the threshhold of damage production. The severity of skin damage and the areas covered are not large, and the data does not preclude the partition model. However, in general, one can state that the results of the concrete surface experiments are best described by employing a vapor transport model.

HD can dissolve in PMMA, which is a thickener used to make THD, and we anticipated that there would be some sorption of the agent into the polymer. As the data reported in Table 4 indicates, the solution/sorption of HD into PMMA did not occur in the time period of these experiments. Even at the 25-mg contamination level, no agent was recovered from the polymer after the alcohol rinse. Similarly, no damage was observed in any test animal, and no verification of either model could be obtained. The same general results were obtained with the nylon fabric. There was no significant sorption of the agent into the fabric, and the animal tests revealed only very mild irritation in the direct contact experiments. No model verification can be made from these results.

5. CONCLUSIONS

It is concluded from these tests that:

- HD sorbs into Plexiglas at a slow rate and can be removed totally with solvent so that a contact hazard no longer exists.

- HD quickly sorbs into concrete, and even solvent rinsing and solvent soaking of this concrete for days does not leach out all of the trapped agent. The remaining agent can be a significant contact hazard especially by direct contact.

- The nylon carrier cloth, XM40, used in these tests sorbed HD slowly; and after 30-min contact, HD was almost completely removed by the solvent rinse. Damage to skin was evident only at the high dose by direct contact, and the damage was confined to a very small area (e.g., 0.1 sq inches).

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REPLY TO
ATTENTION OF

RDCB-DSR-S

JUL 1 1 2016

MEMORANDUM THRU Director, Edgewood Chemical Biological Center, (RDCB-D/ Dr. Joseph Corriveau), 5183 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5424

FOR Defense Technical Information Center, 8725 John J. Kingman Road, Ft Belvoir, VA 22060

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1. This action is in response to an Edgewood Chemical Biological Center (ECBC) Internal Request for a Change in Distribution.

2. The attached listed documents have been reviewed by ECBC Subject Matter Experts and deemed suitable for the change in distribution to read "Approved for public release; distribution unlimited."

3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

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1. Manthie, J.H., Heitkamp, D.H. Dorsey, R.W., Stark, W.C., Bona, D.M., Moore, R.D., and Cameron, K.P., *Mustard Contact Hazard, Correlation of Effects on Skin with Contamination Levels Recovered from Dental Dam and Painted Steel Surfaces*, CRDEC-TR-88142, August 1988 (Dist. B. - U. S. Government Agencies) **DTIC: CBRNIAC-CB-009397**

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